American Journal of Medical Genetics 70:11–15 (1997)

Primary Developmental Field III: Clinical and Epidemiological Study of Blastogenetic Anomalies and Their Relationship to Different MCA Patterns

María Luisa Martínez-Frías[1]* and Jaime L. Frías[2]

[1]*ECEMC and Departamento de Farmacología, Facultad de Medicina, Universidad Complutense, Madrid, Spain*

[2]*Department of Pediatrics, University of South Florida, Tampa, Florida*

Opitz [1993: BD:OAS XXIX (1):3–37] suggested that during blastogenesis the entire embryo constitutes a developmental field, i.e., the primary developmental field. Based on this principle, he postulated that a single "hit," that during late morphogenesis would cause a monotopic malformation, during blastogenesis would produce a polytopic malformation or an association. Lubinsky [1986: Am J Med Genet [Supp1]2:6–16] had stated previously that "since the embryo develops in an integrated manner, organized and differentiating spatially, temporally, and in an epimorphically hierarchical manner, disturbances result in nonrandom patterns of anomalies." He then concluded that "associations are derivatives of causally nonspecific disruptive events acting on developmental fields." These concepts, confirmed by our epidemiological observations [Martínez-Frías, 1994: Am J Med Genet 49:45–51], imply that some associations are, by definition, abnormalities of blastogenesis that is, that their component congenital anomalies are produced by events occurring during the first 4 weeks of development. We present an analysis of the characteristics of blastogenetic anom-alies and their relationship with midline abnormalities, as well as with the schisis and VACTERL associations. Am. J. Med. Genet. 70:11–15, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: developmental field defects; blastogenesis; associations; VACTERL; schisis association; epidemiology

INTRODUCTION

The stages of development from time of fecundation until the end of gastrulation (stage 12, days 27–28) constitute the period known as *blastogenesis.* The main events of this 4-week stage are the formation of the unilaminar embryo, followed by the bilaminar embryo with the amniotic cavity, and the trilaminar embryo with three germ layers that give rise to *pattern formation.* This involves the partition of undifferentiated regions of the embryo (primary field) into areas of specific morphogenetic fates, the *progenitor fields* [Davidson et al., 1995]. The final stage of blastogenesis, comprising mostly the last part of the fourth week, is very complex as it merges into the beginning of organogenesis.

Opitz [1993] suggested that during early blastogenesis the entire embryo constitutes a developmental field, the *primary developmental field*, and that, for this reason, a single "hit" that during late morphogenesis would produce a single anomaly (or monotopic malformation) during blastogenesis may produce an association.

Previously, Lubinsky [1986] had stated that "since the embryo develops in an integrated manner, organized and differentiating spatially, temporally, and in an epimorphically hierarchical manner, disturbances result in nonrandom patterns of anomalies." He then concluded that "associations are derivatives of causally nonspecific disruptive events acting on developmental fields." From an epidemiological standpoint, we demonstrated [Martínez-Frías, 1994] that, in fact, associations have the highest concurrence of blastogenetic developmental field defects (DFD) among all multiple congenital anomaly (MCA) patterns. The concepts outlined above and our observations imply that associations are, by definition, abnormalities of blastogenesis and, consequently, that their component congenital anomalies are produced during the first 4 weeks of development.

Here we present an analysis of the characteristics of blastogenetic malformations and their relationship with midline defects, as well as with the schisis and VACTERL associations.

Contract grant sponsor: Fundación ONCE of Spain; Contract grant sponsor: Dirreción General de Salud Pública, Ministerio de Sanidad y Consumo of Spain.

*Correspondence to: Dr. M.L. Martínez-Frías, ECEMC, Facultad de Medicina, Universidad Complutense, 28040 Madrid, Spain.

Received 24 August 1994; Accepted 30 August 1996

MATERIALS AND METHODS

The study was based on 20,891 liveborn malformed infants identified by the Spanish Collaborative Study of Congenital Malformations (ECEMC). This is a hospital-based case-control study and surveillance system. All children born in about 62 participating hospitals from all over Spain are examined by physicians who, being interested in the problem of congenital defects, collaborate with the ECEMC program and follow its unique and strict methodology. Children are examined during the first 3 days of life to identify major and/or minor defects. For each case, the next nonmalformed infant of the same sex born in the same hospital is selected as a control subject. Once the case and control infants have been identified, the same physician interviews the mothers of case and control infants to gather information on family history, obstetrical data, and prenatal exposures. In many instances, photographs, karyotypes, imaging studies, pathology reports, and results of other studies are also available for review.

Between April 1976–June 1993, the ECEMC registered a total population of 1,074,029 liveborn infants. Among them, 20,891 were selected as cases because they had major and/or mild defects and some minor anomalies detected during the first 3 days of life.

As described previously [Martínez-Frías, 1995], the ECEMC data base is structured using a relational system which distributes the collected information from each child in several tables related by an identification number which is specific for each infant. The system can be expanded by adding information through another table related by any of their fields to the existing ones. Using this capability, we established a table including 171 codes for blastogenetic defects. Detailed descriptions of the ECEMC methodology were published elsewhere [Martínez-Frías, 1995; Martínez-Frías et al., 1991; Martínez-Frías and Urioste, 1994].

We considered as blastogenetic malformations only those defects that are produced during the first 4 weeks of gestation (Table I). We recognize that some malformations occurring during organogenesis may at times originate during blastogenesis. However, since we cannot identify them clinically, we have not included them in this group. From a statistical standpoint, the final effect, if any, of not incorporating these defects in the blastogenetic group would be to influence the results towards the null hypothesis.

To study the characteristics of blastogenetic defects and their relationship with different well-known patterns of anomaly, the 20,891 malformed children were separated into two groups (Table II): one including the 2,004 infants who presented at least one blastogenetic defect, and the other, the 18,887 babies who did not have any blastogenetic defect. The former group was then separated into two categories, one comprised of children with only blastogenetic defects (1,340), and the other, by those with blastogenetic defects plus other nonblastogenetic anomalies, whether major or minor/mild (664). Using the second and third coding sublevel of our coding system [Martínez-Frías, 1994], we analyzed the patterns of anomaly observed in the different study groups, according to their clinical presentation.

TABLE I. Blastogenetic Defects Included in Present Study

Conjoined twins
Acardia-acephalus twinning
Otocephaly
Atelencephaly-aprosencephaly
Anencephaly
Encephalocele
Spina bifida
Holoprosencephaly
Anophthalmia
Anotia
Amelia
Anomalies of the body stalk/wall
Limb-body wall complexes
Ectopia cordis
Pentalogy of Cantrell
Conotruncal septation defects
DiGeorge sequence
Diaphragmatic defects
Tracheal agenesis
Lung agenesis
Tracheoesophageal fistula
Esophageal duplication
Agenesis of the stomach
Intestinal duplication
Agenesis of the gallbladder
Accessory gallbladder
Renal agenesis
Agenesis of the adrenal glands
Bladder duplication
Exstrophy of bladder
Exstrophy of cloaca
Anal atresia
Sirenomelia
Caudal regression
Sacrococcygeal teratoma
Acrorenal field defect
Polyasplenia field defect
Situs inversus
Axial mesodermal dysplasia complex
Vertebral segmentation anomalies
Spondylocostal/thoracic dysostosis

RESULTS

Table II shows that 2,004 (9.59%) of the 20,891 malformed infants had one or more blastogenetic defects. Of these, 1,340 (66.87%) had only blastogenetic defects, while the remaining 664 (33.13%) had, in addition, other nonblastogenetic anomalies. Table III summarizes the characteristics of the study population in terms of sex ratio, twinning, neonatal death, parental consanguinity, and the existence of first-degree relatives with any type of defect. Infants were divided into three groups: those with at least one blastogenetic defect (with or without other nonblastogenetic defects), those without blastogenetic anomalies, and a nonmalformed control group. Of all the variables, only sex ratio appeared similar in infants with and without blastogenetic defects. The rest were all statistically different ($P < 0.0001$) among the three groups.

Table IV depicts the proportion of children with and without blastogenetic defects who had any midline defect, any of the anomalies considered as components of the schisis association (neural tube defects (NTD), cleft

TABLE II. Study Population: Number and Proportion of Malformed Children With and Without Blastogenetic Anomalies

Malformed infants		With only blastogenetic defects	With blastogenetic plus other defects	Without blastogenetic defects	Total	
					N	%
With blastogenic defects	N (%)	1,340 (66.87)	664 (33.13)	—	2,004 (100)	9.59
Without blastogenic defects	N	—	—	18,887	18,887	90.41
Total	N (%)	1,340 (6.41)	664 (3.18)	18,887 (90.41)	20,891 (100)	100

lip and/or cleft palate, diaphragmatic hernia, and omphalocele), or any of those included in the VACTERL association (vertebral, anal, cardiac, tracheoesophageal, renal, and limb malformations). Among the children with at least one blastogenetic defect, 93.86% had one or more midline defects, while the percentage observed among the infants without blastogenetic defects was 23.24% ($P < 0.0000001$). In Table IV we also show that 51.30% of infants with blastogenetic anomalies had one or more of the defects observed in the schisis association, as compared to only 5.91% ($P < 0.0000001$) among malformed children without blastogenetic anomalies. Similarly, 47.16% of children with blastogenetic defects had one or more of the VACTERL association components with any degree of clinical expression. These malformations were observed only in 8.95% ($P < 0.0000001$) of infants without blastogenetic defects.

DISCUSSION

Close to 10% of liveborn malformed infants have at least one blastogenetic defect. These defects of blastogenesis, as predicted by Opitz [1993], are highly lethal, show no altered sex ratio, have low recurrence risk, include a high proportion of twins, and overwhelmingly affect the midline. We previously demonstrated [Martínez-Frías, 1995] that, as expected, these are also characteristics of midline defects. Our data (Table IV) show that close to 94% of infants with blastogenetic malformations have midline defects. This proportion is essentially identical ($P = 0.08$) in patients with only blastogenetic defect (93.21%), and in those who have both blastogenetic and nonblastogenetic anomalies (95.18%).

In the so-called schisis association, the proportion of children with malformations of blastogenetic origin is much lower, i.e., 51.30%. This is due to the fact that although all components of the schisis association are midline defects, not all of them can always be classified as being of blastogenetic origin (e.g., oral clefts, omphalocele). This is further reflected in the highly significant difference ($P < 0.0000001$) found between the proportion of schisis associations (Table IV) in children with only blastogenetic anomalies (57.91%) and in those with blastogenetic and nonblastogenetic defects (37.95%). The same is true, but in the opposite direction, for the VACTERL association (35.22% and 71.23%, respectively), indicating that those defects included in the VACTERL association are frequently observed in infants with MCA pattern of nonblastogenetic defects. Moreover, we observed children with VACTERL anomalies among patients without blastogenetic defects. This is due to the fact that sometimes any of the VACTERL anomalies clinically may appear as being of nonblastogenetic origin (such as renal dysplasia). However, the difference between the propor-

TABLE III. Characteristics of Study Groups: Global Results*

Characteristics		Malformed infants[a]		
		With blastogenetic defects	Without blastogenetic defects	Control group[a]
Sex[b]	M	1,037	9,878	541,833[c]
	F	892	8,991	511,297
	M/F	1.16[d]	1.10[e]	1.06
Twins	No	1,978	18,451	20,266
	Yes	71	251	163
	%	3.59	1.34	0.80
Neonatal death	No	1,250	18,108	20,351
	Yes	695	520	32
	%	35.73	2.79	0.16
Consanguinity	No	1,837	17,820	19,641
	Yes	80	533	397
	%	4.17	2.90	1.98
First-degree relatives with any defects	No	1,897	18,227	18,277
	Yes	171	2,093	490
	%	9.01	11.48	2.61

*All comparisons except d with e are statistically significant ($P < 0.0001$).
[a]We used cases with specified data.
[b]Differences with total cases are due to intersex.
[c]Total livebirths.

TABLE IV. Proportion of Children in Study Groups With at Least One Midline Defect, Any Component Defect of the Schisis Association, or Any of Those Included in the VACTERL Association

		Total number (%)	With at least one defect[a]		
			Midline	Schisis	VACTERL
With blastogenetic defects					
Only with blastogenetic defects	N	1,340	1,249	776	472
	(%)	(100)	(93.21)*	(57.91)*	(35.22)*
With blastogenetic plus other defects	N	664	632	252	473
	(%)	(100)	(95.18)*	(37.95)*	(71.23)*
Total with at least one blastogenetic defect	N	2,004	1,881	1,028	945
	(%)	(100)	(93.86)*	(51.30)*	(47.16)*
Without blastogenetic defects	N	18,887	4,390	1,116	1,690
	(%)	(100)	(23.24)	(5.91)	(8.95)

*Differences with the group of infants without blastogenic defects are statistically significant ($P < 0.0000001$).
[a]Some children are included in more than one group.

tion of children with VACTERL anomalies among patients with at least one blastogenetic defect (47.16%) and among patients without blastogenetic defects (8.95%) is highly significant ($P < 0.0000001$). The proportion of VACTERL anomalies observed in the group of patients with nonblastogenetic defects is higher than in those with the schisis association.

It is possible that conditions with blastogenetic defects and cleft lip and/or palate may be caused by chromosomal abnormalities, single gene defects, or long-term-acting environmental agents. The situation for omphalocele, a defect that has been considered nonblastogenetic in origin, is different, since it is significantly ($P = 0.000002$) more frequently associated with blastogenetic defects (1.2% among infants with blastogenetic defects, and 0.5% among infants without blastogenetic anomalies). This may indicate that this defect may be induced during blastogenesis, possibly through an alteration of morphogenetic movements.

Opitz [1993, 1994] defined associations as "true biological entities that represent the idiopathic occurrence of multiple congenital anomalies during blastogenesis." Our observations demonstrate that indeed most of the VACTERL and so-called schisis associations are of blastogenetic origin.

Opitz [1993] also stated that all blastogenetic malformations constitute a dysmorphogenetic response of the primary developmental field, i.e., of the whole embryo during early blastogenesis. Thus, according to this definition, associations (which he defines as "idiopathic occurrence of multiple congenital anomalies during blastogenesis") should be considered the dysmorphogenetic reaction of the primary developmental field. As such, if the damage occurs during early blastogenesis, the result may be a severe polytopic primary developmental field defect (DFD). If the damage occurs during the formation of the progenitor fields, or during the latter part of blastogenesis, it may produce a polytopic or monotopic DFD, which may also be less severe. This implies that different events occurring during blastogenesis may lead to broad variability in the expression of different types of malformations and associated malformations.

A defining characteristic of developmental field defects is their causal heterogeneity. Therefore, in order to conclude that blastogenetic associations are the dysmorphogenetic response of the primary developmental field, one needs to demonstrate that they are heterogeneous. This is, indeed, the case, as demonstrated in the study of selected associations [Khoury et al., 1983; Martínez-Frías, 1994]. However, it has been said that heterogeneity cannot be proven because most of the well-known associations are sporadic in nature. We hypothesize that, since they are highly lethal, they may produce miscarriages more frequently than nonblastogenetic defects, thus masking their recurrence at birth. To confirm this point, we looked at the proportion of gestations ending in abortions in mothers of infants with blastogenetic defects and in controls. The proportion was 11.41% (429 abortions/3,760 gestations) in the former, and 9.56% (3,241 abortions/33,917 gestations) among mothers of control infants. The difference was statistically significant ($P \ll 0.00001$). This shows that mothers of infants with blastogenetic defects have a higher number of pregnancies which end in spontaneous abortions than mothers of control infants, and this may indicate a higher recurrence risk of these lethal blastogenetic associations. Thus, low recurrence risk at birth does not necessarily imply that these associations are always sporadic. This is also in agreement with the high proportion (9.01%) of cases with first-degree relatives with any type of defect that we observed in the group of infants with blastogenetic defects (Table III).

In conclusion, from the results of this epidemiological analysis it appears that associations are indeed constituted by defects of blastogenesis, and that those defects of blastogenesis represent, as suggested by Opitz [1993], the dysmorphogenetic response of the primary developmental field. As such, they are highly lethal, show no altered sex ratio, include a high proportion of twins, and affect the midline.

ACKNOWLEDGMENTS

We thank all the collaborators of the ECEMC who collected information. This work was supported in part by a grant from Fundación ONCE of Spain and by a grant from the Dirección General de Salud Pública, Ministerio de Sanidad y Consumo of Spain.

REFERENCES

Davidson EH, Peterson KJ, Cameron RA (1995): Origin of bilateria body plans: Evolution of developmental regulatory mechanisms. Science 270:1319–1325.

Khoury MJ, Cordero JF, Greenberg F, James LM, Erickson JD (1983): A population study of the VACTERL association: Evidence for its heterogeneity. Pediatrics 71:815–820.

Lubinsky (1986): Current concepts: VATER and other associations: Historical perspectives and modern interpretations. Am J Med Genet [Suppl] 2:6–16.

Martínez-Frías ML (1994): Developmental field defects and associations: Epidemiological evidence of their relationship. Am J Med Genet 49:45–51.

Martínez-Frías ML (1995): The primary developmental field I: Clinical and epidemiological characteristics. Am J Med Genet 56:372–381.

Martínez-Frías ML, Urioste M (1994): Segmentation anomalies of the vertebrae and ribs: A developmental field defect. Epidemiologic evidence. Am J Med Genet 49:36–44.

Martínez-Frías ML, Frías JL, Rodríguez-Pinilla E, Urioste M, Bermejo E, Cereijo A, Gayá F (1991): Value of clinical analysis in epidemiological research: The Spanish registry experience. Am J Med Genet 41:192–195.

Opitz JM (1993): Blastogenesis and the "Primary Field" in Human Development. New York: Alan R. Liss, Inc., for the National Foundation—March of Dimes. BD:OAS XXIX (1):3–37.

Opitz JM (1994): Associations and syndromes: Terminology in clinical genetics and birth defects epidemiology: Comments on Khoury, Moore, and Evans. Am J Med Genet 49:14–20.